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# Significance of lipopolysaccharide detection in children with pulmonary infections

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**Abstract. – OBJECTIVE: The present study aimed to observe the alterations of lipopolysaccharide (LPS) and some other laboratory indexes in children suffering from pulmonary infections, and to investigate the condition of Gram-negative bacterial infection.**

**PATIENTS AND METHODS: All the patients received routine blood test, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), Mycoplasma pneumoniae antibody IgM (MP-IgM), LPS, blood culture and chest X-ray examination. The clinical data was collected followed by induction arrangement and statistical analysis.**

**RESULTS: In terms of the rate of abnormity in peripheral white blood cell count and positive rate of blood CRP, no significant difference was found between children with pulmonary infections and the healthy individual in the control group ($p > 0.05$). The positive rates of blood MP-IgM were 33.33% and 32.26% in children with different progressive stages of pulmonary infections, which were significantly lower than those in the control group (62.96%) ($p < 0.05$). The positive rates of blood LPS in the observation group were higher than those in the control group, especially for those children at progressive stages within one week; and the difference between them was significant ($p < 0.05$). With regard to blood bacterial culture, the positive rates were 9.52% and 29.03% for children in progressive stages within one week and over one week in the observation group, respectively; the latter was significantly higher than that in the control group ($p < 0.05$). The result of the correlation analysis suggested a weak correlation between the positive rate of increased blood LPS in the observation group and that in blood bacterial culture ($\chi^2 = 6.61$, $p < 0.05$; Pearson's contingency coefficient C = 0.34). However, there was no significant correlation between the positive rate of increased blood LPS and peripheral blood white cell count, CRP, or MP-IgM ($p > 0.05$).**

**CONCLUSIONS: Endotoxemia is often accompanied by pulmonary infections, and gram-negative bacterium is a common pathogenic bacterium in children with different progressive stages of pneumonia.**

*Key Words:*
Pulmonary infection, Lipopolysaccharide, Endotoxemia.

## Introduction

Pediatric pneumonia is a serious disease with high morbidity rate that threatens children's health in China. A significant increase in Gram-negative bacterial infection among children with pneumonia is noted. However, it is impossible to accurately make the etiological diagnosis in 20% to 60% of cases due to the difficulty in obtaining pathogen[1]. Lipopolysaccharide (LPS), the main component of endotoxin, is one of the major pathogenic factors of bacteria, which is essential in diagnosis of Gram-negative bacterial infection[2].

## Patients and Methods

### General Information

A retrospective analysis was conducted in 52 cases of children with pulmonary infections who were hospitalized in the Department of Pediatrics from January, 2012 to December, 2012, including 33 males and 19 females aging between three months and ten years old within the progressive stage of two days to 30 days (21 cases within two days to seven days and 31 cases over 8 days). During the course of disease, 34 cases were accompanied by fever and 18 cases did not have this symptom. Based on clinical manifestations and chest X-ray examination results on admission, 49 patients were involved with bronchopneumonia and 3 patients were involved with solid changes of pulmonary lobe and segment, accompanied by 15 cases of



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wheezing onset, 3 cases of congenital heart disease, one case of pneumothorax, 2 cases of pleural effusion, one case of pulmonary atelectasis, one case of heart failure, one case of cytomegalovirus infection, 3 cases of anemia, and one case of disseminated intravascular coagulation (DIC). All the patients were inquired with medical history and allergic history in detail after admission and subjected for routine physical examination, laboratory and chest X-ray examinations for excluding the possibilities of foreign body in bronchus, tuberculosis and allergic disease. Another 27 cases of children without bacterial infectious diseases and clinical manifestation who were hospitalized during the same period, were enrolled into the control group, including 18 males and 9 females aging between three months and thirteen years old within the progressive stage of one day to 15 days (23 cases within one day to seven days and 4 cases over 8 days). They were diagnosed with upper respiratory infection in 5 cases, viral encephalitis in 7 cases, muggy syndrome in one case, convulsion in 2 cases, anaphylactoid purpura in 3 cases, acute urticaria in 5 cases, trauma in 2 cases, tetany caused by vitamin D deficiency on admission in one case, and cerebral infarction in 2 cases.

### Methods

All the patients were subjected for routine blood test, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), *Mycoplasma pneumoniae* antibody IgM (MP-IgM), β-lipopolysaccharide (LPS), blood culture and chest X-ray examination. The clinical data was collected followed by induction arrangement and statistical analysis.

### Statistical Analysis

SAS8.1 statistical software was used for statistical analysis. $\chi^2$ test (row × column) was used for analyzing the difference between the observation group and control group and the difference in the positive rate of various laboratory indexes in different courses of disease in the observation group. $\chi^2$ test with data in bidirectional disorderly R × C table and Pearson's contingency coefficient C were used for the analysis of the relevance and closeness between LPS and positive rate of other clinical indexes in the observation group. $p < 0.05$ was regarded as statistically significant.

## Results

### Alteration in Laboratory Indexes

Among 52 cases in the observation group, 27 cases were found with abnormal white blood cell count (51.92%), including 21 cases (40.38%) with increased white blood cell count, and 6 cases with reduced white blood cell count; There were 5 cases (9.62%) in progressive stages within 7 days and one case (1.92%) in the progressive stage over 7 days. Among the patients in the control group, 11 cases were found with increased peripheral white blood cell count and one case with reduced peripheral white blood cell count. There was no significant difference in peripheral white blood cell count or positive rate of increased blood CRP between patients at different progressive stages in the observation group and control group ($p > 0.05$) (the percentage of increased neutrophils was not included in the statistics due to the lack of uniform standards as the percentage of neutrophils for children at different ages was different). However, the degree of change was much higher than that in the control group. The positive rates of blood MP-IgM were 33.33% and 32.26% in patients with different progressive stages of pulmonary infections, which were significantly lower than those in the control group (62.96%) ($\chi^2 = 4.14$ *vs* 5.47; $p < 0.05$). Among the patients in the observation group, 14 cases (26.92%) were found with increased LPS, including 7 cases (33.33% in this progressive stage) in progressive stages within 7 days and 7 cases (22.58% in this group) over 7 days. The difference between two groups was not statistically significant. The percentage of increased LPS in progressive stages within 7 days was significantly higher than that in the control group ($\chi^2 = 5.21$, $p < 0.05$). The percentage of increased LPS in progressive stages over 7 days was higher than that in the control group, but the difference was not significant ($\chi^2 = 2.53$, $p > 0.05$). With regard to blood bacterial culture, among 11 cases (21.15%) in the observation group, 2 cases were confirmed with Gram-positive bacteria and 9 cases (81.82%) with Gram-negative bacteria. There were 2 cases (9.52%) and 9 cases (29.03%) at different progressive stages. The positive rate of patients in progressive stages over 7 days was significantly higher than that in the control group ($\chi^2 = 6.49$, $p < 0.05$) (Table I). The positive rates were 9.52% and 29.03% for children in progressive stages within one week and over one week in the obser-

**Table I.** Comparison of positive cases and positive rate of patients in two groups.

| Laboratory index | Observation group | | | Control group |
|---|---|---|---|---|
| | n | ≤ 7d | > 7d | |
| n 52 | 21 | 31 | 27 | |
| WBC (↑/↓) | 27 (51.92%) | 13 (61.90%) | 14 (45.16%) | 12 (44.44%) |
| CRP (↑) | 22 (42.31%) | 11 (52.38%) | 11 (35.48%) | 14 (51.85%) |
| MP-IgM (+) | 17 (32.69%) | 7 (33.33%) | 10 (32.26%) | 17 (62.96%) |
| LPS (↑) | 14 (26.92%) | 7 (33.33%) | 7 (22.58%) | 2 (7.41%) |
| Blood culture (+) | 11 (21.15%) | 2 (9.52%) | 9 (29.03%) | 1 (3.70%) |

vation group; and the latter was significantly higher than that in the control group ($p < 0.05$).

### Correlation Analysis

$\chi^2$ test with data in bidirectional disorderly R × C table revealed a weak correlation between increased blood LPS and the positive results of blood culture in the observation group ($\chi^2 = 6.61$, $p < 0.05$; Pearson's contingency coefficient C = 0.34). However, there was no significant correlation between the positive rate of increased blood LPS and peripheral blood white cell count, blood CRP, or MP-IgM ($p > 0.05$) (Table II).

## Discussion

LPS derived from the outer membrane of cell wall of Gram-negative bacteria is the main component of endotoxin, and widely distributed in polluted air, organic powders, etc. A series of clinical symptoms, such as asthma and bronchopneumonia, can be caused or aggravated after inhalation of certain concentration of the above substances.

Gram-negative bacteria infection is often accompanied by the presence of endotoxemia. Endotoxin is an important promoter to mediate Gram-negative bacterial sepsis. Endotoxemia plays an important role in the occurrence and development of pneumonia. Endotoxin can induce the synthesis and release of various cytokines in the host and stimulate a series of body's pathophysiological changes in combination with its receptors or regulatory proteins. LPS can activate C/EBP in mouse lung after lung deposition, which plays a vital role in the production of tumor necrosis factor-α, IL-6, and macrophage inflammatory protein-2[3]. A study conducted on 48 cases of patients with Gram-negative bacterial pulmonary infections by Ming et al[4] demonstrated that the blood endotoxin and CRP levels were significantly higher in the patients than those in the normal control group. The blood endotoxin level in the moderate to severe infection group was higher than that in the mild infection group. The difference in CRP level between two groups was not significant. A bronchial pneumonia model was established by injection of LPS into rats by Hou et al[5], who demonstrated significant signs of inflammation in rat bronchi and lung tissues. Zeng et al [6] revealed that LPS could cause a dose-dependent increase of lipocalin-2 (LCN2) expression level in mouse lung tissue, promote the production of interleukin-8 (IL-8) in respiratory tract epithelium, and mediate neutrophil chemotaxis and excessive release of inflammatory mediators, which was associated with the degree of inflammatory pathological injury of lung tissue to a certain extent.

**Table II.** Analysis of the correlation and closeness between LPS and other laboratory indexes (cases).

| ZDT | WBC | | CRP | | MP-IgM | | Blood culture | |
|---|---|---|---|---|---|---|---|---|
| | + | – | + | – | + | - | + | – |
| + | 6 | 7 | 5 | 9 | 4 | 9 | 7 | 8 |
| – | 20 | 19 | 17 | 21 | 14 | 25 | 5 | 32 |
| $\chi^2$ | 0.103 | | 0.34 | | 0.11 | | 6.61 | |
| Contingency coefficient C | – | | – | | – | | 0.34 | |

The blood LPS level in children with pneumonia in our case load is significantly higher than that in the control group, especially for those in progressive stages over 7 days. Among 11 cases of children with positive results of blood bacterial culture, 9 cases (81.82%) were confirmed with Gram-negative bacteria. The correlation analysis suggested a weak correlation between blood LPS level and the positive rate of blood bacterial culture in the patients. The reasons may include the following: (1) The patients with pneumonia admitted in our hospital were mostly transferred from other hospitals after ineffective treatment, so their courses of disease were relatively longer. The laboratory indexes showed atypical changes after the treatment in the local hospitals. The initial condition thus could not be accurately estimated by blood CRP, ESR and WBC count. In this study, the differences in peripheral blood WBC count and blood CRP between the observation group and control group were not significant, which could be the result from then ineffective treatment; (2) The lower positive rate of blood culture was caused by the interference of colony in the early treatment. And the actual flora species and positive rate of blood culture, thus, were difficult to be accurately determined; (3) The diagnosis of pneumonia was mainly based on clinical manifestations and imaging studies. The possibility of pathogen infection other than bacterium was not excluded.

Endotoxin is a kind of potent pyrogen[7]. Endotoxemia may be one of the contributing factors for immunocompromised patients with fever of unknown reason. It has been reported in China that 67.74% of adult patients with fever were accompanied by endotoxemia. About 50% of Gram-negative bacilli were detected in patients with bacterial pneumonia caused by nosocomial infection, with the fatality rate up to 30%-40%. Ren et al[1] conducted a study on the culture of bronchoalveolar lavage fluid taken from 368 cases of children with refractory pneumonia. Among patients with positive bacterial culture, 5.1% was confirmed with Gram-positive bacteria, 24.6% with Gram-negative bacteria, 69.7% with bacterial parasites, 73.2% with mixed infections. The cases of patients with simultaneous mycoplasma pneumoniae infections were the most.

There were 34 cases of children suffered from pneumonia with fever in our case series, accounting for 65.39% of cases in this group, including 11 LPS-positive cases (32.35%), which were higher than that in the control group. The positive rate of blood culture in pneumonia group was 21.15%, of which the proportion of Gram-negative bacteria was up to 81.12%. This is likely to be caused by a better curative effect of primary anti-infective drug on Gram-positive bacteria but a poor effect on Gram-negative bacteria according to the requirements of antibiotic hierarchical management by the Ministry of Health. The especially high positive rate of blood culture for children in progressive stages over 7 days indicated a greater chance of drug resistance or secondary Gram-negative bacterial infection with the increased course of disease, which may be also one of the important reasons of prolonged course for the disease. In addition, the number of cases of patients with increased LPS in progressive stages within 7 days was higher, but the positive rate of blood culture was lower. This is likely to be associated with excessive anti-infection treatment prior to admission, relatively sensitive pathogenic bacteria in early stage during the short course of disease, and relatively less drug resistance or suprainfection.

The immune response plays an important role in the development of pneumonia caused by Gram-negative bacteria. LPS was used to process mouse splenic antigen-presenting cells (APCs) and tubular epithelial cells (RCs) in a a study done by Eleftheriadis et al[8], in which they demonstrated the overexpression of subunits of immunoproteasome LMP7 on APCs and RCs. The proteasome can be converted into immune proteasome during the inflammation, and an autoimmune response of $CD8^+$ T cells can be induced by the generated peptide pool. Bacterial permeability-increasing protein (BPI) and lipopolysaccharide binding protein (LBP) are members of lipid transfer protein/LPS-binding protein family, which play vital roles in the innate immune response of Gram-negative bacteria[9]. The amino acid sequence of Sb-BPI/LBP1 may contain a N-terminal BPI/LBP/CETP domain BPI1 with three functional zones displaying LPS binding activity, which exists in all normal tissues including the liver, adductor muscle, heart and blood cells. The general up-regulation at 24 hours after excitation by LPS indicates that Sb-BPI/LBP1 is a constitutive and inducible acute phase protein that can promote the host to produce immune defense against Gram-negative bacterial infections. Previous studies[10] revealed that lipopolysaccharide (LPS) induced endothelial dysfunction by activating the immune system. When lack of im-

mune cells, LPS is able and sufficient to promote the fibrosis of endothelial cells by the activity-dependent mechanism of activin receptor-like kinase 5 (ALK5). LPS can induce the expression of fibroblast-specific protein in endothelial cells and up-regulation of secretion of extracellular matrix protein, accompanied by down-regulation of endothelial markers. The pneumonia caused by Gram-negative bacteria is usually clinically characterized by its severe condition and prolonged course. In addition to the influence of cytokines induced by endotoxin, immune response may be also an important factor affecting the development of the disease.

Soop et al[11] have reported that LPS could increase the levels of platelet microparticle (PMP), mononuclear microparticle (MMP) and endothelial microparticle (EMP) (MPs aresome membrane-bound vesicles smaller than 1 m that are produced by activated and dead cells), affect their phenotypes including nuclear contents, and promote inflammation and thrombosis. Particles may be the source of high mobility group protein B1 (HMGB1) and other nuclear molecules in the blood during the inflammatory reaction.

It was reported that in addition to Gram-negative bacteria, *Mycoplasma* and *Chlamydia* that can directly produce toxins, toxins in the blood of the patients with pneumonia caused by other pathogenic bacteria, were derived from the intestinal tract[7]. An analysis of blood WBC count, C-reactive protein (CRP), LPS and *Mycoplasma pneumoniae* antibody in 34 cases of adult patients with *Mycoplasma pneumoniae pneumonia* (MPP) was conducted by Han[12] and observed a rise of endotoxin level in most of MPP patients. However, the elevated blood endotoxin level was not significantly correlated to the titer of *Mycoplasma pneumoniae* antibody. A synthetic lipopeptide MALP-2 was reported by Galanos et al[13], which was derived from mycoplasma fermentation and had a strong expression of endotoxin-like activity. Its toxicity is induced through Toll-like receptor 2 (Tlr2) and equal to lethal toxicity of LPS.

The present study demonstrated that, up to 32.69% of the patients were identified with positive results of *Mycoplasma pneumoniae* in the observation group. However, there was no correlation between LPS and MP-IgM ($p>0.05$). It still requires further verification whether the previously reported LPS increase in MPP children is derived from this lipopeptide. In addition, in this study, the positive rates of blood MP-IgM in children with pneumonia in different courses were lower than those in the control group, which is likely to be associated with the high carrying rate of *Mycoplasma pneumoniae* (MP) in the crowd. About 20% to 40% of children infected with MP have been reported. This antibody could not be detected in some patients due to the long hospitalization time. However, those who were with relatively short course of disease (around one week) in the control group were found to have high level of IgM. Therefore, the possible effect of antibiotics and glucocorticoid used prior to admission cannot be excluded.

Based on the role of LPS in the occurrence and development of pneumonia, how to suppress LPS effect using effective methods and to reduce its damage to the body during anti-infective therapy, has become a research hotspot worldwide. After pulmonary infection, LPS induces mRNA and protein expressions of inducible nitric oxide synthase (iNOS) by the transcription-dependent process in alveolar epithelial cells, regulates NO production, and plays an important role in host defense and inflammation, which is not dependent on ATP signal. LPS-induced iNOS expression is mediated by calcium-dependent PKC$\alpha$-$\beta$1[14]. It was reported by Zhang et al[15] that TMEM16A expressed in rat alveolar type epithelial cells (AT- ) and human lung epithelial cell line A549 inhibited the activation of LPS-induced nuclear factor B (NF- B), reduced the release of pro-inflammatory cytokines such as TNF-$\alpha$ and IL-8, and prevented acute lung injury. The activation of peroxisome proliferator-activated receptor $\alpha$ (PPAR$\alpha$) using ligands including WY-14643 can inhibit the increase of LPS-induced pro-inflammatory cytokines and stress level of nitrogen oxides, and reduce LPS-induced acute lung injury[16]. NADPH oxidase can limit the transcriptional activity of LPS-induced NF- B and expression of pro-inflammatory cytokines, and limit LPS-induced lung inflammation and injury[17]. It has been reported that alkannin[3], limonene[18], coxtex moutan[19] and esculentoside[20] have significant effects on relieving LPS-induced acute lung injury.

In recent years, more and more studies have reported the Gram-negative bacterial infections. Therefore, rapid diagnosis of Gram-negative bacterial infection and the subsequent endotoxemia has become a hotspot in clinical research, as the current bacteriological examination requires a longer time. Moreover, the excessive use of antimicrobial drugs causes a decline in the positive

rate of blood culture. Since the results can be obtained by LPS assay within 2 hours, it is conducive to early determination of the type of bacterial infection in the presence or absence of endotoxemia. It can also be used as a reference index to measure the severity and to determine the prognosis, or used as one of the indexes of clinical treatment and,to determine the clinical efficacy, or used for drug screening. In addition, based on the study using an animal model of LPS-induced acute lung injury, exhaled breath condensate (EBC) collection may be a valuable tool in monitoring the markers such as nitrogen oxides and $H_2O_2$, which can shed the light on the role of environmental pollution in respiratory diseases[21].

## Conclusions

LPS detection method is simple, rapid and accurate, and also of important significance in clinical selection of antibiotics for timely treatment, which further accelerates patient's recovery.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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